CARBONYLS IN OXIDIZING FAT. II. THE IDENTITY AND AMOUNTS OF STEAM VOLATILE MONOCARBONYLS IN A RANCID FREEZER-STORED PORK FAT

A. M. GADDIS AND REX ELLIS

Meat Laboratory

Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture Beltsville, Maryland

A considerable amount of reasearch on the carbonyl compounds of oxidized fat has been done by investigators of autoxidative mechanisms and reversion of vegetable oils. This work has been thoroughly reviewed by Morris *(11)* and Lea *(10)*. Conventional methods were used in the investigations and results tended to be qualitatively and quantitatively incomplete. Swift, O'Connor, Brown, and Dollear *(14)* in 1949 isolated hexanal, 2-octenal, and 2,4-decadienal from the steam distillate of autoxidized cottonseed oil. The monocarbonyl classes represented by these compounds have since proven to be the major ones present in autoxidized fat.

In recent years the use of 2,4-dinitrophenylhydrazone[a] derivatives, and the application of improved chromatographic methods has given a more complete indication of the monocarbonyls present in a product. Forss, Dunstone, and Stark *(2, 3)* in 1955, using adsorption chromatography, isolated acetone, n-saturated aldehydes C_2 and C_6, alk-2-enals C_3 and C_5–C_{11}, and 2,4-dienals C_5–C_{11} in oxidized skim milk. Recently, Pippen, Nonaka, Jones, and Stitt *(13)* identified acetone, methyl ethyl ketone, n-saturated aldehydes C_2–C_6 and C_8 and C_9, alk-2-enals C_5–C_7 and C_{10} and C_{11}, alk-2,4-dienal C_7, and diacetyl in 2,4-DNPH derivatives obtained by passing air through a simmering mixture of chicken muscle tissue and water.

The methods employed by Forss, Dunstone, and Stark *(2, 3)* and Pippen, Nonaka, Jones, and Stitt *(13)*, although having certain advantages, require relatively large quantities of 2,4-DNPH derivatives, and are laborious and time-consuming. Moreover, as shown by Forss and Dunstone *(4)* decomposition and rearrangement of the 2,4-DNPH's may occur during lengthy exposure to the adsorbents used, silicic acid and silica gel. Gaddis and Ellis *(6)* and Ellis, Gaddis, and Currie *(1)* have reported rapid paper chromatographic methods of separating monocarbonyl 2,4-DNPH's into classes and the classes into individual compounds. These micro-methods have been applied by Gaddis, Ellis, and Currie *(7)* in a preliminary study of changes taking place in the proportions of classes of the volatile monocarbonyls of freezer-stored pork tissue fat. Gaddis and Ellis *(5)* identified hexanal, propanal, ethanal, methanal, acetone, and methyl ethyl ketone in the saturated classes of a very rancid pork fat. The purpose of this paper is to report the kinds and relative amounts of steam volatile monocarbonyls in a cooked and an uncooked pork tissue fat in the early stages of rancidity.

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[a] Hereinafter referred to as 2,4-DNPH.

EXPERIMENTAL

Solvents, reagents, materials, and equipment. These items were the same as were used in similar operations described in earlier papers (1,5,6,7).

Materials and methods. The tissue fat sample chosen was an uncured bacon side stored at 15° F. for 15 months. The fatty tissue was ground and rendered under mild conditions (5,7). Determinations were made on 10.0 g. samples of the unheated and heated fat. The fat was heated at 165° C. for 15 minutes to approximate cooking. Steam distillation, collection of the carbonyls as 2,4-DNPH's, extraction of the derivatives, and separation into monocarbonyl and dicarbonyl fractions was done as described in previous papers (5,7). The total monocarbonyls were estimated by their absorbance at the wavelength of maximum absorption, and resolved into classes by the paper chromatographic method reported in (6) as applied by Gaddis et al. (7). The separated classes were extracted from the paper, and proportions were determined in the manner described by the latter authors (7). Paper chromatographic separation of the classes into individual compounds was done by the method of Ellis et al. (1) and Gaddis and Ellis (6). The individual compounds were extracted from the paper and identified by comparison of R_F values with those of authentic compounds, non-separation or difference in R_F when mixed with authentic DNPH's, maxima in carbon tetrachloride, fading rate in alcoholic alkali of the major maxima, and the presence, dissappearance, or absence of secondary maxima around 520–535 mμ (1,8). Authentic C_5–C_{10} alk-2-enal and C_3–C_{12} alk-2,4-dienal derivatives were furnished by Dr. E. L. Pippen, of our Western Utilization Research and Development Laboratory. Other 2,4-DNPH's were prepared as previously described (1,6).

RESULTS AND DISCUSSION

The freezer-stored bacon side used in this study, when ground and mixed, had a peroxide value of 17. Samples from it had been tested by the method of Moser et al. (12) at monthly intervals up to 11 months of storage, at which time it was judged still in the good range in aroma and acceptable range in flavor. When this study was made after 15 months of storage, the flavor of the uncooked rendered fat was in the objectionable range, and the cooked in the unpleasant category. The cooked sample had a much stronger and somewhat fishy flavor.

The absorbance values in 100 ml. of carbon tetrachloride of the monocarbonyl and dicarbonyl fractions from the unheated sample were 0.87/346–9 mμ and 0.19/375–85 mμ. Values for similar fractions from the heated sample were 1.41/361–5 mμ and 0.27/349–52 mμ. These shifts in the maxima of heated mono- and dicarbonyls have been noted and discussed by Gaddis, Ellis, and Currie (7).

Table 1 shows the results obtained in the separation of the monocarbonyls into classes. These values result from the combined spots from three paper strips read in 3.00 ml. of carbon tetrachloride. Amounts of the monocarbonyl solutions (100 ml.) spotted on each strip were 1.724 ml. for the unheated and 1.063 ml. for the heated. These amounts were calculated from the formula,

$$x\ ml. = \frac{1.5}{Absorbance}$$

(7). The total amount of material spotted for each monocarbonyl fraction should have an absorbance value of 1.499 in 3.00 ml. of carbon tetrachloride. However, in the unheated, recovery at 346 mμ was 0.953, and in the heated at 361 mμ, it was 1.229. This appears to indicate recoveries of 63.57% for the unheated and 81.99% for the heated. It has been noted (1, 6) that losses are due to failure of some material to move from the origin, and incomplete

TABLE 1

Separation by paper chromatography of monocarbonyls into classes[1]

λmμ	Unheated fat absorbance values			Heated fat absorbance values		
	Spot			Spot		
	1	2	3	1	2	3
320	0.398	0.102		0.237	0.155	
330	0.544	0.128		0.330	0.225	
340	0.660	0.162		0.402	0.312	
343	0.670	0.170		0.414	0.336	
346	0.680[2]	0.180	0.093	0.420	0.358	0.305
349	0.674	0.184	0.097	0.420	0.380	0.335
352	0.655	0.188	0.102	0.410	0.400	0.365
355		0.188	0.106	0.392	0.415	0.400
358		0.188	0.114	0.370	0.425	0.430
361		0.184	0.117	0.335	0.430	0.464
365		0.177	0.120		0.430	0.490
370			0.125		0.415	0.525
375			0.125		0.390	0.550
380			0.121			0.565
385			0.117			0.570
390						0.554

[1] Combined spots from 3 paper strips in 3.00 ml. CCl₄.
[2] Figures enclosed in rectangles denote maximum absorption.

extraction from the paper. Recovery has been consistently observed to be better, the higher the concentration of carbonyls. This may be due to the presence of non-carbonyl impurities which would have a greater influence at higher volumes of material spotted. Based on the absorbance at the maxima (7), the data indicated 68.0% n-alkanal class, 19.0% mixture of classes, and 13.0% alk-2,4-dienal class in the unheated. In the heated, proportions were 29.6% n-alkanal, 30.3% alk-2-enal, and 40.0% alk-2,4-dienal. The group in the unheated monocarbonyls, which is composed of a mixture of classes, has been referred to in early work (5, 7).

Each class was resolved into individual compounds by paper chromatography (1, 6). For identification, spots were cut out from paper strips and extracted. The resulting fractions were identified by comparing R_F values run singly and mixed with authentic derivatives, and their absorption maxima in carbon tetrachloride and/or alcoholic alkali. Spectra in alcoholic alkali were useful in examining fractions separated on Vaseline impregnated paper. Although Vaseline can be removed from the fractions (1), an impurity which absorbs at the lower wavelengths carries over. This interfered, particularly when the fractions were small. The fading rate in alcoholic alkali (1, 8) is also very helpful in clearing up doubtful cases. Spectrophotometric values obtained for authentic members of the four classes are shown in Table 2.

Identification is greatly simplified by the effectiveness of the separation into classes (6). 2-Alkanone, n-alkanal, alk-2-enal, and alk-2,4-dienal are separated completely except for the acetone derivative which separates in the n-alkanal class, and methanal and ethanal in the alk-2-enal class. When the

TABLE 2

Spectrophotometric constants of carbonyl classes

Monocarbonyl classes	CCl₄ λ max.	Alc. KOH Major λmax.		Minor λ max.
	mμ	mμ	Fading at 30 min.	mμ
2-Alkanones				
C₃ – C₉	349–52	430–34	1.2%	535
n-Alkanals				
C₁	330	420–6	69.0%	none
C₂ – C₃	343	430	42.0%	520
C₄ – C₁₄	343–6	430–4	27.0%	520
Alk-2-enals				
C₃	355	454	7.0%	none
C₄	361	454	2.8%	none
C₅ – C₁₂	361–5	456–60	1.7%	none
Alk-2,4-dienals				
C₅	370–5	470–4	1.9%	none
C₆	380	474–80	0.0%	none
C₇ – C₁₂	380	480–4	0.0%	none

classes are resolved into individual compounds, acetone forms mixtures with propanal and butanal, and ethanal with acrolein.

The identification of the monocarbonyls of the unheated and the heated fat is shown in Tables 3 and 4. In Table 3, the n-alkanal class contained propanal, hexanal, and nonanal; and the mixed class is shown to be composed of methanal and ethanal, three unknown compounds and $C_7 - C_{11}$ alk-2-enals. The constants of ethanal indicated that acrolein was not present. The first unknown compound with R_F 0.55 had spectra similar to n-alkanals but no secondary maximum in alcoholic alkali. The fading rate was also less than it should be for such a type of compound. Moreover, no n-alkanal would be expected in this position. The other two unknowns had properties intermediate between n-alkanals and alk-2-enals (5, 7). No 2-alkanone would be present in these positions. These are apparently compounds of a different class, possibly unconjugated unsaturated compounds; although mixtures with C_5 and C_6 alk-2-enals might be involved. A similar group of three unknown compounds has been found in more oxidized fat (7). Constants of propanal indicated the presence of little, if any, acetone. Traces of acetone, methyl ethyl ketone, methanal, and ethanal have been previously found (5, 7) as the result of benzene extraction following the carbon tetrachloride extraction. In this instance, however, no carbonyls were detected in the benzene extracts. The 2,4-dienal class contained the C_7, C_9, C_{10}, and C_{11} compounds. Identification of the carbonyls in the heated fat, shown in Table 4, presented no problems. The three unknown compounds, methanal, ethanal, propanal, and nona-2,4-dienal, present in unheated fat, were not detected in the heated sample. However, octanal was present in the heated fat, and was not observed in the unheated fat.

Table 5 shows the amounts of the classes and individual compounds found in 10.0 g. of fat in terms of absorbance, micro-moles, and micro-mole percent. The absorbancies of the classes are corrected values for monocarbonyls in

TABLE 3

Carbonyls in unheated fat

Unknowns R_F	CCl$_4$ λ max. mμ	Alcoholic λ max. mμ	KOH λ sec. max. mμ	Fading at 30 min.	R_F authentic compounds	Kind of carbonyl/DNPH	Agreement w/constants of authentic
Saturated class							
Propylene glycol							
0.63	343–6	430–4	500–10[1]	24.5%	C_8–0.64	n-alkanal	yes
0.92	346	430–4	500–10[1]	25.0%	C_6–0.92	n-alkanal	yes
Vaseline system							
0.55	346	430–4	500–10[1]	24.0%	C_8–0.55	n-alkanal	yes
Mixed class							
Propylene glycol							
0.21	330				C_1–0.21	2-enal	yes
0.38	343				C_2–0.38	2-enal	yes
0.55	343–6	428–36	none	16.4%	C_4–0.54	2-enal	no
0.71	352–5	440–48	none	7.0%	C_5–0.73	2-enal	no
0.80	352–5	444–50	none	6.1%	C_6–0.80	2-enal	no
Vaseline system							
0.68		450–58	none		C_7–0.68	2-enal	yes
0.62		450–58	none		C_8–0.62	2-enal	yes
0.57		450–58	none		C_9–0.57	2-enal	yes
0.52		450–58	none		C_{10}–0.52	2-enal	yes
0.42		450–58	none		C_{11}–0.42	2-enal	yes
2,4-Dienal class							
Propylene glycol							
0.70	380	465–80	none		C_7–0.70	2,4-dienal	yes
Vaseline system							
0.58	375–80	465–80	none		C_9–0.58	2,4-dienal	yes
0.52		465–80	none		C_{10}–0.52	2,4-dienal	yes
0.47		465–80	none		C_{11}–0.47	2,4-dienal	yes

[1] Disappeared after 30 min.

TABLE 4

Carbonyls in heated fat

Unknowns R_F	CCl₄ λ max. mμ	Alcoholic λ max. mμ	KOH λ sec. max. mμ	Fading at 30 min.	R_F authentic compounds	Kind of carbonyl/ DNPH	Agreement w/constants of authentic
Saturated class							
Propylene glycol 0.91	346–9	430–9	500–10 [1]	25%	C_6–0.91	n-alkanal	yes
Vaseline system							
0.64		432	500–10 [1]	25%	C_8–0.64	n-alkanal	yes
0.58		432	500–10 [1]	25%	C_9–0.58	n-alkanal	yes
2-enal class							
Vaseline system							
0.73		452–6	none		C_7–0.73	2-enal	yes
0.67		452–6	none		C_8–0.67	2-enal	yes
0.60		452–6	none		C_9–0.61	2-enal	yes
0.52		452–6	none		C_{10}–0.52	2-enal	yes
0.45		452–6	none		C_{11}–0.46	2-enal	yes
2,4-dienal class							
Propylene glycol 0.68	380	464–75	none		C_7–0.68	2,4-dienal	yes
Vaseline system							
0.59	380	464–80	none		C_{10}–0.59	2,4-dienal	yes
0.52		464–75	none		C_{11}–0.52	2,4-dienal	yes

[1] Disappeared after 30 min.

TABLE 5

Quantitative relationships of monocarbonyls

	Unheated fat						Heated fat					
	Corr. absorbance 10 g.	Percent based on absorbance	Micromoles per 10 g.	Percent micromoles (total)	Rel. parts absorbance	Micromoles per 10 g.	Corr. absorbance 10 g.	Percent based on absorbance	Micromoles per 10 g.	Percent micromoles (total)	Rel. parts absorbance	Micromoles per 10 g.
n-alkanals	0.642	72.3	3.01	82.2			0.487	29.6	2.28	39.8		
Methanal					0.100	0.022						
Ethanal					0.234	0.052					—	
Propanal					0.400	0.089						
Hexanal					12.86	2.808					12.97	2.133
Octanal					———	———					0.347	0.058
Nonanal					0.178	0.039					0.542	0.089
Unknowns	0.029	3.2	X	X								
1					0.063							
2					0.085							
3					0.072							
Alk-2-enals	0.107	12.1	0.37	10.1			0.498	30.3	1.74	30.4		
Hepta-2-enal					0.162	0.087					0.155	0.374
Octa-2-enal					0.140	0.076					0.135	0.325
Nona-2-enal					0.125	0.067					0.157	0.376
Deca-2-enal					0.162	0.087					0.141	0.340
Undeca-2-enal					0.097	0.053					0.133	0.325
Alk-2,4-dienals	0.110	12.4	0.38	7.7			0.660	40.1	1.71	29.8		
Hepta-2,4-dienal					0.088	0.010					0.353	0.032
Nona-2,4-dienal					0.110	0.012						
Deca-2,4-dienal					2.362	0.251					18.35	1.659
Undeca-2,4-dienal					0.070	0.007					0.211	0.019
Total	0.888		3.66				1.645		5.73			

100 ml. based on the recoveries (discussed in a preceding paragraph) in the class separations, and the fact that the mixed class from the heated mono-carbonyls was found to be composed of 27.0% n-alkanal and 17.7% unknown class. Micro-moles were calculated on the basis that three micro-moles in 100 ml. of carbon tetrachloride were found to have average absorbancies of 0.640 for n-alkanals, 0.861 for alk-2-enals, and 1.160 for alk-2,4-dienals.

The qualitative and quantitative changes in the carbonyls on heating are of considerable interest. The fate of methanal, ethanal, propanal, the three unknown compounds, and 2,4-nonadienal on heating is not clear. Hexanal, propanal, and ethanal *(5)* have been detected in volatiles trapped during heating. The indication has been *(7)* that the amounts volatilized during heating are relatively very small. However, most of the compounds that disappear in the heated monocarbonyls were originally present only in trace amounts. In earlier experiments with more oxidized fat, propanal has carried over into the heated monocarbonyls *(7)*, but methanal has never been detected under these conditions. It is probable that such disappearances are due to both destruction during heating and volatilization. Hexanal and deca-2,4-dienal *(14)* were predominantly the major compounds in their respective classes in both unheated and heated fat. For instance, on a micro-mole basis, hexanal was 90.3% and 96.9% of the n-alkanal class, and deca-2,4-dienal 89.6% and 97.0% of the alk-2,4-dienal class respectively. This might cause failure to detect some trace amounts of monocarbonyls since the capacity of the filter paper is small. Hexanal was the major monocarbonyl in both the unheated and the heated fat. A similar observation was made by Klose *(9)* for rancid turkey fat. However, hexanal showed a decrease in the heated fat, while deca-2,4-dienal and the other unsaturated monocarbonyls increased considerably. The five alk-2-enals were similar in proportions.

Quantitative data given may be considered tentative in nature. The method for separation into classes is reproducible *(7)*. A mixture containing known quantities of the four classes, methyl ketones 24.6%, n-saturated aldehydes 20.8%, 2-enals 24.1%, and 2,4-dienals 30.5%, was found to contain 25.3%, 24.6%, 24.0%, and 26.1%, respectively *(6)*. Thus, values for 2,4-dienals may be somewhat low and the other classes high. The difficulty arises from the fact that the classes of compounds handled vary in stability. The alk-2,4-dienal hydrazones are particularly sensitive to light and air *(6)*. Further study is required on the quantitative limitations of the procedure used.

SUMMARY

Micro-methods of separating, identifying, and estimating monocarbonyls have been applied to a freezer-stored pork fat in the early stages of rancidity. Trace amounts of acetone and methyl ethyl ketone found in earlier studies with more oxidized pork fat were not observed. Heating at 165° C. influenced not only the amount, but the kind of monocarbonyls detectable. n-Alkanals C_1, C_2, C_3, C_6, C_9, three unknown carbonyls, alk-2-enals C_7, C_8, C_9, C_{10}, C_{11}, and alk-2,4-dienals C_7, C_9, C_{10}, C_{11} were found in the unheated fat. In the heated fat, n-alkanals C_6, C_8, C_9, alk-2-enals C_7, C_8, C_9, C_{10}, C_{11}, and alk-2,4-dienals C_7, C_{10}, C_{11} were observed.

Tentative quantitative determinations indicated the micro-mole content of the identified monocarbonyls. Hexanal and deca-2,4-dienal were the dominant compounds present.

LITERATURE CITED

1. ELLIS, REX, GADDIS, A. M., AND CURRIE, G. T. Paper chromatography of 2,4-dinitrophenylhydrazones of saturated aliphatic aldehydes. *Anal. Chem.*, 30, 475 (1958).

2. FORSS, D. A., DUNSTONE, E. A., AND STARK, W. The volatile compounds associated with oxidized flavour in skim milk. *J. Dairy Research*, 22, 91 (1955).

3. FORSS, D. A., DUNSTONE, E. A., AND STARK, W. Further observations on the volatile compounds associated with oxidized flavour in skim milk. *J. Dairy Research*, 22, 345 (1955).

4. FORSS, D. A. AND DUNSTONE, E. A. Change of 2,4-dinitrophenylhydrazones on alumina and silica gel columns. *Australian J. Chem.*, 10, (4) 506 (1957).

5. GADDIS, A. M. AND ELLIS, REX. Volatile saturated aliphatic aldehydes in rancid fat. *Science*, 126, 745 (1957).

6. GADDIS, A. M. AND ELLIS, REX. Paper chromatography of 2,4-dinitrophenylhydrazones. The resolution of 2-alkanones, alkanal, alk-2-enal, and alk-2,4-dienal derivatives. *Anal. Chem.* 31, 870 (1959).

7. GADDIS, A. M., ELLIS, REX, AND CURRIE, GEORGE T. Carbonyls in oxidizing fat. I. Separation of steam volatile monocarbonyls into classes. *Food Research*, 24, 283 (1959).

8. JONES, L. A., HOLMES, J. C., AND SELIGMAN, R. B. Spectrophotometric studies of 2,4-dinitrophenylhydrazones. *Anal. Chem.*, 28, 191 (1956).

9. KLOSE, A. A. (Unpublished data, 1952).

10. LEA, C. H. Recent developments in the study of oxidative deterioration of lipids. *Chemistry and Industry* (London) 41, 1303 (1953).

11. MORRIS, S. G. Fat rancidity. Recent studies on the mechanism of fat oxidation in relation to rancidity. *J. Agr. Food Chem.* 2, 126 (1954).

12. MOSER, H. A., JOEGER, C. M., COWAN, J. C., AND DUTTON, H. J. The flavor problem of soybean oils. II. Organoleptic evaluation. *J. Am. Oil Chemists' Soc.*, 24, 291 (1947).

13. PIPPEN, E. L., NONAKA, M., JONES, F. F., AND STITT, F. Volatile compounds of cooked chicken. I. Compounds obtained by air entrainment. *Food Research*, 23, 103 (1958).

14. SWIFT, C. E., O'CONNOR, R. T., BROWN, L. E., AND DOLLEAR, F. G. The aldehydes produced during the autoxidation of cottonseed oil. *J. Am. Oil Chemists' Soc.*, 26, 297 (1949).